Role of the Unfolded Protein Response Pathway in Regulation of \textit{INO1} and in the \textit{sec14} Bypass Mechanism in \textit{Saccharomyces cerevisiae}

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\textbf{ABSTRACT}

\textit{INO1}, encoding inositol 1-phosphate synthase, is the most highly regulated of a class of genes containing the repeated element, UAS\textsubscript{INO1} in their promoters. Transcription of UAS\textsubscript{INO1}-containing genes is modulated by the availability of exogenous inositol and by signals generated by alteration of phospholipid metabolism. The unfolded protein response (UPR) pathway also is involved in \textit{INO1} expression and the \textit{ir1}Δ and \textit{hac}1Δ mutants are inositol auxotrophs. We examined the role of the UPR in transmitting a signal generated in response to inositol deprivation and to alteration of phospholipid biosynthesis created in the \textit{sec14}Δ\textit{cki1}Δ genetic background. We report that the UPR is required for sustained high-level \textit{INO1} expression in wild-type strains, but not for transient derepression in response to inositol deprivation. Moreover, the UPR is not required for expression or regulation of \textit{INO1} in response to the change in lipid metabolism that occurs in the \textit{sec14}Δ\textit{cki1}Δ genetic background. Thus, the UPR signal transduction pathway is not involved directly in transcriptional regulation of \textit{INO1} and other UAS\textsubscript{INO1}-containing genes. However, we discovered that inactivation of \textit{Sec14p} leads to activation of the UPR, and that \textit{sec14}\textit{cki1} strains exhibit defective vacuolar morphology, suggesting that the mechanism by which the \textit{cki1} mutation suppresses the growth and secretory defect of \textit{sec14} does not fully restore wild-type morphology. Finally, synthetic lethality involving \textit{sec14} and UPR mutations suggests that the UPR plays an essential role in survival of \textit{sec14 cki1} strains.

\textbf{T\textsc{he} promoters of many yeast phospholipid structural genes contain variants of a 10-bp repeated \textit{cis}-acting promoter element, the inositol-sensitive upstream activating sequence (UAS\textsubscript{INO1}), which controls transcription in response to the soluble precursors inositol and choline (C\textsc{arman} and H\textsc{enry} 1989; G\textsc{reenberg} and L\textsc{opes} 1996). Among the UAS\textsubscript{INO1}-containing genes, the \textit{INO1} gene, encoding inositol 1-phosphate (1-P) synthase (D\textsc{onahue} and H\textsc{enry} 1981), exhibits the most dramatic regulation and is, therefore, frequently used as a reporter for the entire regulon (H\textsc{irsch} and H\textsc{enry} 1986; C\textsc{arman} and H\textsc{enry} 1989; L\textsc{opes} et al. 1991).

Transcription of \textit{INO1} and other UAS\textsubscript{INO1}-containing genes also responds to a signal generated from alteration of phospholipid metabolism (H\textsc{enry} and P\textsc{atton-V\textsc{ogt}} 1998; see Figure 1 for phospholipid metabolic pathways). The \textit{overproduction} of inositol (Opi\textsuperscript{−}) phenotype, indicative of overexpression of the \textit{INO1} gene, is associated with mutants defective in biosynthesis of phosphatidylethanolamine (PE; G\textsc{reenberg} et al. 1983; S\textsc{ummers} et al. 1988; G\textsc{riac} et al. 1996). The fact that the Opi\textsuperscript{−} regulatory phenotype is conferred by mutations in structural genes involved in phospholipid biosynthesis suggests that the regulatory mechanism controlling expression of \textit{INO1} and other UAS\textsubscript{INO1}-containing genes might involve a signal generated by ongoing phospholipid metabolism (H\textsc{enry} and P\textsc{atton-V\textsc{ogt}} 1998). This idea was strengthened when a strong Opi\textsuperscript{−} phenotype was observed in \textit{sec14}\textit{cki1} strains (P\textsc{atton-V\textsc{ogt} et al. 1997). The \textit{SEC14} gene encodes a phosphatidylinositol (PI)/PC transporter essential for viability and secretion (B\textsc{ankaitis} et al. 1989, 1990). At the restrictive temperature, \textit{sec14}Δ mutants arrest at the late Golgi stage of the secretory pathway (N\textsc{ovick} et al. 1980, 1981). Mutations in the CDP-choline pathway for PC biosynthesis (\textit{cki1}, \textit{cct1}, and \textit{ept1}) suppress the growth and secretory defects of \textit{sec14} mutants via a bypass mechanism (C\textsc{leves} et al. 1991). Thus, \textit{sec14}Δ strains carrying these suppressors are viable at the \textit{sec14}Δ restrictive temperature, but they exhibit both Opi\textsuperscript{−} and overproduction of choline (Opc\textsuperscript{−}) phenotypes (P\textsc{atton-V\textsc{ogt} et al. 1997). The Opc\textsuperscript{−} phenotype is directly related to elevated phospholipase D1 activity, which results in increased production of choline and phosphatidic acid (P\textsc{a}; S\textsc{reenivas} et al. 1998). The Opc\textsuperscript{−} phenotype of \textit{sec14}\textit{cki1} strains is eliminated if the \textit{SPO14} (P\textsc{ld1}) gene, which encodes phospholipase D1 (R\textsc{ose} et al. 1995), is deleted (S\textsc{reenivas} et al. 1998). However, the Opi\textsuperscript{−} phenotype is also eliminated when \textit{SPO14/PLD1} is deleted (S\textsc{reenivas} et al. 1998). The fact that the Opi\textsuperscript{−} phenotype and elevated

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**INO1** expression are dependent upon Pld1p supports the hypothesis that a signal related to PA production is responsible for **INO1** induction in **sec14** mutant strains elevated to the restrictive temperature (Patton-Vogt et al. 1997; Henry and Patton-Vogt 1998; Sreenivas et al. 1998). Furthermore, active Pld1p is essential for the **sec14** bypass mechanism and **sec14** mutant strains fail to grow at the **sec14** restrictive temperature (Sreenivas et al. 1998; Xie et al. 1998).

The unfolded protein response (UPR) signal transduction pathway also influences **INO1** expression. Under endoplasmic reticulum (ER) stress, Ire1p, a transmembrane kinase spanning the ER membrane, is activated and carries out specific endoribonucleaseolytic cleavage of **HAC1** mRNA (Cox et al. 1993; Mori et al. 1993; Shamu and Walter 1996; Sidrauski and Walter 1997; Rüegsegger et al. 2001). Only the spliced form of the **HAC1** transcript is known to be effectively translated (Chapman and Walter 1997; Kawahara et al. 1997), and consequently Hac1p is detectable only in UPR-activated cells (Cox and Walter 1996). Hac1p functions as a basic leucine zipper (bZIP) transcription factor by binding to the downstream activating sequence (DAS) of target genes known as UPREs (unfolded protein response elements; Mori et al. 1992; Cox and Walter 1996). UPREs were originally found in promoters of chaperone family genes such as **KAR2**, **PDI1**, and **EUG1** (Mori et al. 1992; Kohno et al. 1993). A single UPRE element is sufficient to activate transcription from a heterologous promoter in response to the accumulation of unfolded proteins in the ER lumen (Cox et al. 1993). In addition to being defective in UPR activation, *ire1Δ* and *hac1Δ* mutants require exogenous inositol for growth and express low levels of **INO1** transcript (Nikawa and Yamashita 1992; Cox et al. 1993, 1997; Mori et al. 1993). Cox et al. (1997) reported that under inositol-depleting conditions, the UPR is activated and suggested that the activation of the UPR leads to expression of **INO1** in response to inositol deprivation. However, Mori et al. (2000) reported that Hac1p produced from an unspliced form of **HAC1** mRNA is able to suppress inositol auxotrophy of **hac1Δ**, suggesting that activation of the UPR *per se* may not be required for **INO1** expression.

In this report, we examine the relationship between the UPR and signals generated from phospholipid metabolism in the **sec14** genetic background. We report that a functional UPR is not necessary for **INO1** activation or regulation in the **sec14** genetic background. However, a functional UPR is required for the bypass mechanism by which the **chi1Δ** mutation suppresses the secretory defect of **sec14** mutants.

### MATERIALS AND METHODS

**Strains, media, and growth conditions:** The genotypes and sources of strains used in this study are listed in Table 1. All of the yeast strains listed in Table 1 are of the W303 genetic background. Strains were constructed by standard tetrad analysis (Sherman et al. 1978; Ross et al. 1990). The HCY399 and HCY400 triple mutants containing **sec14** and **hac1Δ** in conjunction with **ire1Δ** or **hac1Δ** were generated by crosses of SHY653 containing **sec14** **hac1Δ** with JCY147 or JCY408 containing **ire1Δ** or **hac1Δ**. To ensure a uniform genetic background, HCY399 and HCY400 were then backcrossed to the wild-type strains, SHY629 and SHY652, respectively. HCY006, HCY029, HCY030, HCY031, and HCY032 were obtained as spores from the cross between HCY400 and SHY652. HCY401, HCY402, HCY403, and HCY404 were obtained as spores from the cross between HCY399 and SHY629. HCY136 was obtained as a spore colony from a cross between JPV110 and HCY006. Two sets of four strains from each cross (HCY401–HCY404 and HCY029–HCY032, each set derived from a single tetratype ascus) were used to assess the growth phenotypes. Rich YEPD (yeast extract, peptone, dextrose), synthetic complete, synthetic minimal, and mutants require exogenous inositol for growth and expression are dependent upon Pld1p supports the hypothesis that a signal related to PA production is responsible for **INO1** induction in **sec14** mutant strains elevated to the restrictive temperature (Patton-Vogt et al. 1997; Henry and Patton-Vogt 1998; Sreenivas et al. 1998). Furthermore, active Pld1p is essential for the **sec14** bypass mechanism and **sec14** mutant strains fail to grow at the **sec14** restrictive temperature (Sreenivas et al. 1998; Xie et al. 1998).

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inositol but lacking a specific amino acid, adenine, or uracil) was used to test auxotrophic requirements. To select for deletion mutations carrying the kanMX6 marker, 200 mg/liter geneticin (YEPD + G418; Calbiochem, La Jolla, CA) was added to the media described above. To test tunicamycin (tm) sensitivity, 1 mM tunicamycin (Boehringer Mannheim, Indianapolis) was added to I\(^+\), I\(^-\), and YEPD media (COX and WALTER 1996). To analyze growth phenotypes of yeast strains, cells were cultured in YEPD or I\(^+\) liquid medium to the mid-logarithmic phase of growth. Cells were collected by centrifugation and washed twice with sterile distilled water (dH\(_2\)O). The cells were then adjusted to OD\(_{600}\) of 0.7 with sterile dH\(_2\)O. The cells were initially diluted 1:100 using dH\(_2\)O followed by 1:10 serial dilutions. From each dilution 10 \(\mu\)l of cells were spotted on an appropriate plate and allowed to grow at the designated temperature.

Synthetic lethal analysis: To test the genetic interaction between the sec14 and hac1 mutations, JV110 was crossed to HCY006. Diploids were sporulated and dissected. All resulting spores contained the deletion mutation chik\(\Delta\), which has no effect on viability, but suppresses the growth defect conferred by sec14\(\Delta\). The genotypes of viable spores were determined by their ability to grow on amino-acid drop-out media and YEPD + G418. Once the genotypes of viable spores were determined, the tetrads were categorized into parental ditype, nonparental ditype, and tetratype with respect to the sec14\(\Delta\) and hac1\(\Delta\) mutations, and the genotypes of spores that failed to germinate were deduced. In the initial cross, ~75% of spores containing the sec14\(\Delta\) chik\(\Delta\) hac1\(\Delta\) genotype failed to germinate, whereas the sec14\(\Delta\) chik\(\Delta\) segregants exhibited >90% viability. The relatively low viability of the sec14\(\Delta\) chik\(\Delta\) hac1\(\Delta\) segregants suggested that this genotype might be inviable except in the presence of a suppressor segregating in the cross. To test this hypothesis, a sec14\(\Delta\) chik\(\Delta\) spore colony (HCY136) was selected from a tetrad that contained a viable sec14\(\Delta\) chik\(\Delta\) hac1\(\Delta\) segregant, and the cross with HCY006 was repeated. Analysis of the synthetic lethality of sec14\(\Delta\) and hac1\(\Delta\) was assessed as described above in a cross between HCY136 and HCY006.

\(\beta\)-Galactosidase assays: For INO1-CYC-lacZ expression assays, yeast strains harboring a leu2 or ura3 mutation were transformed to leucine or uracil prototrophy with the autonomously replicating plasmids, pMR1036 (RUIN-NORIEGA 2000) or pHS59 (LOPES et al. 1991), using the standard lithium acetate method (HILL et al. 1991). The transformants were pregrown in I\(^+\) medium to mid-logarithmic phase. Cells were collected by centrifugation, washed with sterile dH\(_2\)O, and diluted to OD\(_{600}\) of 0.1 in repressing (I\(^-\)) or derepressing medium (I\(^+\)), and allowed to grow at the designated temperature. Samples of 1 ml were taken at the designated time points and immediately frozen at ~80°C. For analysis, samples were thawed on ice, suspended in 1 ml of sterile dH\(_2\)O, and analyzed for \(\beta\)-galactosidase activity using yeast \(\beta\)-galactosidase assay kit (Pierce, Rockford, IL). For UPRE-CYC-lacZ expression assays, the strains containing the integrated UPRE-CYC-lacZ (Leu) or 2 \(\mu\)m UPRE-CYC-lacZ (Ura) reporter construct (COX and WALTER 1996) were precultured overnight at 25°C and diluted to OD\(_{600}\) of 0.1 in YEPD medium. The cells were then shifted to 30°C and 37°C, and samples were collected at the designated time points. The samples were processed as described above.

Tests for Opip\(^-\) and Opcc\(^-\) phenotypes: The method for detection of the Opip\(^-\) phenotype has been described previously (GREENBERG et al. 1983; SWEDEN and co-workers 1992). Strains were patched onto I\(^-\) liquid medium and allowed to grow at 30°C for 2 days. The plates were then sprayed with a suspension of a diploid tester strain (AID), which is homozygous for inol1 and ade1. The cells were incubated at 30°C for another 2 days. The Opcc\(^-\) test was performed in a similar fashion, except that the media used in this assay were either I\(^-\) or I\(^+\), lacking choline, and the tester strain was a choline auxotroph, cho2 opl3 (PATTON-YOGI et al. 1997).

Lipid analysis: Strains were grown in I\(^+\) medium at 30°C, harvested at mid-logarithmic phase of growth by centrifugation, washed twice with sterile dH\(_2\)O, resuspended in 5 ml of I\(^-\) medium to OD\(_{600}\) of 0.25, and allowed to grow for 1 or 4 hr at 30°C. A total of 100 \(\mu\)Ci of [\(^3\)P]orthophosphate/ml was then added to I\(^-\) medium, and the cells were incubated for 20 min. The cells were harvested and treated with trichloroacetic acid. Labeled lipids were extracted as previously described (ATKINSON et al. 1980). The individual phospholipid species were resolved by two-dimensional paper chromatography (STEINER and LESTER 1972) and quantified by PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Electron microscopy: Cells subjected to electron microscopy were processed by the procedure of WEBB et al. (1997), with modifications. This method is specifically optimized for visualizing vacuolar structures. Other structures such as the ER may not be conspicuous. Briefly, the cells were grown in a 5-ml culture of YEPD to an OD\(_{600}\) of ~0.5. The cells were diluted to OD\(_{600}\) of 0.1 in YEPD and shifted to 37°C. Samples of 5 ml were taken at 0, 1, and 2 hr after the temperature shift. Each sample collected was fixed for 2 hr at 30°C by the addition of 3% glutaraldehyde and 5 mM CaCl\(_2\) buffered with 100 mM sodium cacodylate, pH 6.8. The cells were collected by centrifugation, washed once in 0.1 M K\(_2\)HPO\(_4\) adjusted to pH 5.8 with citric acid, and 1.2 M sorbitol, and incubated in the same solution for 10 min at 37°C. The cells were then washed once in 0.1 M K\(_2\)HPO\(_4\) adjusted to pH 5.8 with citric acid, and 1.2 M sorbitol. The cells were resuspended in 0.5 ml of the same buffer. A total of 50 \(\mu\)l of \(\beta\)-glucuronidase-type H-2 (114,000 units/ml, Sigma, St. Louis) and 2.5 mg of Zymolyase 20T (20,000 units/g, Seikagaku, Tokyo) was added. The cells were incubated for 2 hr at 30°C to allow the cell wall to be removed. After washing three times with 100 mM sodium cacodylate buffer containing 5 mM CaCl\(_2\), the cells were postfixed for 30 min at room temperature in 1% OsO\(_4\), 1% K-ferrocyanide, and 5 mM CaCl\(_2\) buffered with 100 mM sodium cacodylate, pH 6.8. The samples were washed four times with dH\(_2\)O, resuspended for 5 min in 1% thiocarbohydrazide in dH\(_2\)O, washed four times with dH\(_2\)O, and fixed for 5 min in 1% aqueous OsO\(_4\). The samples were washed four times with dH\(_2\)O and dehydrated through a series of ethanol dilutions (50, 70, 80, 90, and 100%), followed by two washes with 100% propylene oxide. The samples were infiltrated in a (1:1) propylene oxide:LR White resin mixture for several hours, transferred to 100% LR White resin, and infiltrated overnight at room temperature. The next day, a fresh change of 100% LR White resin was added, and infiltration was extended for an additional 8 hr. The resin was polymerized in gelatin capsules at 60°C for 24 hr. Thin (80-nm) sections were cut using a DDK diamond knife on a Reichert-Jung Ultracut E. The sections were placed on copper grids, stained with Reynolds lead citrate, viewed, and photographed in a Hitachi 7100 transmission electron microscope operated at an acceleration voltage of 50 keV.

RESULTS

The hac1\(\Delta\) and ire1\(\Delta\) mutations do not confer inositol auxotrophy in the sec14\(\Delta\) chik\(\Delta\) genetic background: Triply mutant strains, sec14\(^{+}\) chik\(\Delta\) ire1\(\Delta\) and sec14\(^{+}\) chik\(\Delta\) hac1\(\Delta\), were created by standard genetic crosses, as described in MATERIALS AND METHODS. Tetratype spore colonies from single tetrads derived from crosses of HCY399 to
TABLE 1

Yeast strains used in this study

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<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<td>SHY629</td>
<td>MATa his3 trp1 ura3</td>
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</tbody>
</table>

ShY629 and HCY400 to SHY652 were tested for growth on I+ and I- medium and for Opi- and OpC- phenotypes. The genotypes of the analyzed spore colonies were the following wild type (HCY403), ire1A (HCY401), sec14ts chi1A (HCY402), and sec14ts chi1A ire1A (HCY404), or Opi- (HCY032), hac1A (HCY030), sec14ts chi1A (HCY031), and sec14ts chi1A hac1A (HCY029). Detailed genotypes of these strains are listed in Table 1. In addition, we tested related sec14ts, sec14ts ire1A, and sec14ts hac1A strains (HCY636, HCY364, and HCY65; Table 1). All of the strains grew normally in I+ medium at 25°C and 30°C. The sec14ts and hac1A single mutants exhibited slow or defective growth on I- medium (i.e., have an Ino- phenotype; Figure 2A), as previously reported (Nikawa and Yamashita 1992; Cox et al. 1993, 1997; Cox and Walter 1996). The sec14ts mutant grew normally on I- and I- media at 25°C and 30°C and failed to grow on any medium at its restrictive temperature of 35°C or higher, as previously reported (Bankettis et al. 1989, 1990; data not shown). At 33°C, the sec14ts strain exhibited a slightly leaky Ino- phenotype, but grew normally on I- medium (data not shown). In contrast to sec14ts strains, sec14ts ire1A and sec14ts hac1A strains grew poorly on I- media at 33°C and exhibited an Ino- phenotype more stringent than that of the sec14ts strain grown at this temperature (data not shown). Similar to sec14ts chi1A strains, but in contrast to their ire1A and hac1A parents, the sec14ts chi1A ire1A and sec14ts chi1A hac1A triple mutants grew normally on I- media at 25°C and 30°C (Figure 2A). This Ino- phenotype suggests that the INO1 gene is expressed in the sec14ts chi1A ire1A and sec14ts chi1A hac1A genetic background, a topic that will be discussed subsequently. Unexpectedly, the sec14ts chi1A ire1A and sec14ts chi1A hac1A strains failed to grow on any medium, including YEPD, I+, and I- media at 37°C, the restrictive temperature for the sec14ts allele (Figure 2, A and B). This temperature-sensitive phenotype of the triple mutants resembles that of sec14ts strains, as opposed to that of sec14ts chi1A strains (Cleves et al. 1991), and suggests that a negative genetic interaction is occurring between sec14ts and the UPR mutations hac1A and ire1A.

Moreover, both sec14ts chi1A hac1A and sec14ts chi1A ire1A strains exhibited Opi- phenotypes at 30°C, indicative of INO1 overexpression, similar to the phenotype previously observed in the sec14ts chi1A parental strains (Patton-Vogt et al. 1997; Figure 3). The triply mutant strains also exhibited OpC- phenotypes (data not shown), similar to the sec14ts chi1A parent (Patton-Vogt et al. 1997), suggesting that the mutation in the UPR does not affect the elevated level of Pld1p (Spo14p) activity in the sec14ts chi1A genetic background.
Figure 2.—The sec14Δ ckilΔ ire1Δ and sec14Δ ckilΔ hac1Δ strains are able to grow on I− medium at 25° and 30°, but fail to grow at 37°. (A) Two sets of strains, each derived from a single tetrad (HCY029–032 and HCY401–404), were spotted as serial dilutions on I− and I+ medium and incubated at 25°, 30°, and 37° for 4 days. (B) Serial dilutions of the same set of strains were spotted on YEPD medium and incubated at 25°, 30°, and 37° for 4 days.
INO1 is expressed and regulated by inositol in the sec14Δ cki1Δ genetic background in the absence of a functional UPR pathway: To assess INO1 expression in the triple mutants, wild-type, hac1Δ, sec14Δ cki1Δ, and sec14Δ cki1Δ hac1Δ strains were transformed with the INO1-CYC-lacZ reporter gene, as described in MATERIALS AND METHODS. Cells were first grown under repressing conditions (1⁺) at the sec14Δ permissive temperature of 25°C and then shifted to derepressing (1⁻) conditions at 30°C or 37°C (semipermissive and restrictive temperatures, respectively, for sec14Δ). Following a shift from 1⁺ at 25°C to 1⁻ medium at 30°C, hac1Δ and ire1Δ cells exhibited significant initial derepression of INO1, reaching a maximum level after ~4 hr that approached 60–70% of the level achieved by wild-type cells under identical conditions. Within 5 hr following the shift to 1⁻ medium, however, β-galactosidase expression from the INO1 reporter construct in hac1Δ and ire1Δ cells plateaued at a level ~40% of that observed in wild-type cells (Figure 4; ire1Δ data not shown). This pattern of INO1 expression is similar to that reported by Cox et al. (1997) for INO1 expression in ire1Δ cells. Similar to wild-type cells, hac1Δ and ire1Δ cells did not express INO1 at all in 1⁺ medium (Figure 4). Thus, despite the overall decrease in INO1 expression in the hac1Δ and ire1Δ mutants compared to wild-type cells in 1⁻ medium, the mechanism of regulation of INO1 in response to inositol appears to be intact in the absence of a functional UPR.

INO1 expression in sec14Δ cki1Δ cells was found to be similar to patterns reported by Patton-Vogt et al. (1997). In general, INO1 expression levels were found to be higher in sec14Δ cki1Δ cells grown in 1⁻ medium at 25°C, 30°C, and 37°C than in wild-type cells grown under identical conditions and the degree of INO1 overexpression tended to increase with temperature, as previously reported (Patton-Vogt et al. 1997; Figure 4). At the sec14Δ restrictive temperature of 37°C, sec14Δ cki1Δ cells expressed INO1 even in the presence of inositol (Patton-Vogt et al. 1997; Figure 4), whereas wild-type cells did not express INO1 at any appreciable level, at any temperature, in the presence of inositol.

The pattern of INO1 expression in the sec14Δ cki1Δ hac1Δ strain at 25°C and 30°C was similar to the pattern observed in the sec14Δ cki1Δ parent. When shifted from 1⁺ medium at 25°C to 1⁻ medium at 30°C, the sec14Δ cki1Δ hac1Δ strain achieved a level of INO1 expression ~2.5-fold higher than that of the wild-type control grown under identical conditions, while the sec14Δ cki1Δ strain expressed a level ~2.9-fold higher than that of wild type (Figure 4). This high level of INO1 expression is in contrast to the low level of expression observed in the hac1Δ single mutant (Figure 4). INO1 expression patterns were similar in sec14Δ cki1Δ ire1Δ (data not shown). These results are consistent with the growth (Figure 2A) and Opi⁻ phenotypes of the sec14Δ cki1Δ hac1Δ and sec14Δ cki1Δ ire1Δ strains (Figure 3) and confirm that an intact UPR is not required for INO1 derepression and overexpression in the sec14Δ cki1Δ genetic background. Furthermore, since INO1 is not expressed in sec14Δ cki1Δ hac1Δ and sec14Δ cki1Δ ire1Δ strains grown in 1⁺ medium at 30°C (Figure 4; sec14Δ cki1Δ ire1Δ data not shown), the mechanism of regulation in response to inositol also appears to be intact in the absence of a functional UPR in the sec14Δ cki1Δ genetic background at the sec14Δ semipermissive temperature of 30°C.

Phosphatidylinositol synthesis is compromised in hac1Δ and ire1Δ mutants, but not in sec14Δ cki1Δ ire1Δ strains:

Since inositol serves as a precursor to the synthesis of PI, the effect of UPR mutations on phospholipid synthesis was assessed. Wild-type, hac1Δ, ire1Δ, and sec14Δ cki1Δ
Figure 4.—Induction of the INO1-CYC-lacZ reporter gene in wild-type (SH652), sec14Δ cki1Δ (HCY402), hac1Δ (HCY030), and sec14Δ cki1Δ hac1Δ (HCY400) cells. A schematic diagram depicting the construction of pMR1036 is shown above the graph. Plasmid pMR1036 contains a fusion of two UASINO elements derived from INO1, a minimal CYC1 promoter element, and the coding sequence for the Escherichia coli β-galactosidase enzyme. Expression of β-galactosidase from the INO1-CYC-lacZ reporter gene (pMR1036), and transformants were shifted from I/H11001 to I/H11002 (o) or I/H11002 (o) medium at the indicated temperatures. Samples were taken 5 hr following the shift. β-Galactosidase activity was measured as described in MATERIALS AND METHODS. The β-galactosidase activity unit was defined as OD420/min/ml.

ire1Δ strains were pulse labeled with [32P]orthophosphate ([32P]H3PO4), as described in MATERIALS AND METHODS and Table 2. Cells were grown to logarithmic phase in I+ medium at 30° and shifted to I− medium at 30°. They were then incubated for an additional 1 or 4 hr and then labeled for 20 min with [32P]H3PO4. As a control, cells grown in I+ medium were shifted for 1 hr and labeled for 20 min. When cells were pulse labeled in I+ medium at 30°, the phospholipid labeling pattern was similar in all strains (Table 2) and similar to labeling patterns previously described (Atkinson et al. 1980; Patton-Vogt et al. 1997). During the 20-min pulse-labeling period in I+ medium, the wild-type strain incorporated >90% of lipid-associated 32P into PI (Table 2). Also comparable to previously published reports (Kelley et al. 1988), in wild-type cells shifted to I− medium, the relative incorporation into PI declined dramatically to ~3% of total incorporation, while label accumulated in the precursors, PA and cytidine-diphosphate diacylglycerol (CDP-DG). The labeling pattern of other phospholipids was largely unaffected.

The ire1Δ and hac1Δ strains exhibited a labeling pattern similar to wild type when pulse labeled 1 hr following the shift to I− medium. Within 4 hr after the shift to I− medium, however, the proportion of label incorporated into PI in wild-type cells recovered to ~9% of the total label incorporated into phospholipids, while the proportion of label accumulated in PI in the ire1Δ and hac1Δ strains remained low (Table 2). The failure of PI synthesis to recover in ire1Δ and hac1Δ cells correlates with the failure to achieve and sustain a high level of INO1 expression in the absence of a functional UPR (Figure 4).

The proportion of label associated with CDP-DG also remained significantly higher in hac1Δ cells shifted to I− medium, suggesting that CDP-DG, the immediate
preursor of PI, was accumulating to a greater extent in hac1Δ cells than in wild-type cells grown in 1+ medium. However, in hac1Δ cells as compared to wild type, PA labeling declined somewhat and PC labeling increased, indicating that there may be subtle differences in many aspects of lipid metabolism in UPR mutants as compared to wild type. The labeling of PI in the sec14Δ cki1Δ ire1Δ strain shifted to 1− medium recovered in a fashion similar to that seen in the wild-type strain. However, as expected, the proportion of label incorporated in PC in the triple mutant was lower than that in wild type due to the cki1Δ mutation, which blocks PC synthesis via the CDP-choline pathway. PLD-mediated turnover of PC is also elevated in sec14Δ cki1Δ strains, a factor that also influences PC labeling (Patton-Vogt et al. 1997; Sreenivas et al. 1998).

The UPR and sec14 mutations exhibit synthetic lethality: The failure of the sec14Δ cki1Δ ire1Δ and sec14Δ cki1Δ hac1Δ strains to grow at 37° (Figure 2, A and B) suggested the possibility of a negative genetic interaction involving sec14 in combination with hac1Δ or ire1Δ mutations. To test this hypothesis, a cross of a sec14Δ cki1Δ strain to a cki1Δ hac1Δ strain was conducted, as described in Materials and Methods.

As a control, sec14Δ cki1Δ and cki1Δ spo14Δ strains were crossed to each other, since functional phospholipase D1 encoded by SPO14 (PLD1) is known to be required for the viability in strains carrying sec14Δ in combination with cki1Δ or other bypass suppressors (Patton-Vogt et al. 1997; Sreenivas et al. 1998; Xie et al. 1998). Thirty tetrads were dissected from a cross of sec14Δ cki1Δ to cki1Δ spo14Δ and, as expected, no spores of the sec14Δ cki1Δ spo14Δ genotype survived (data not shown), whereas the survival rates of all other genotypes exceeded 90%. We also tested for synthetic lethality between spo14Δ and hac1Δ or ire1Δ. The double mutants, hac1Δ spo14Δ and ire1Δ spo14Δ, were found to be viable and exhibited normal growth (data not shown).

From the diploid generated by the cross of sec14Δ cki1Δ to cki1Δ hac1Δ, 67 tetrads were dissected. While the survival rate of the cki1Δ and cki1Δ hac1Δ spores was 100% and the survival rate of the sec14Δ cki1Δ spores was >90%, only five sec14Δ cki1Δ hac1Δ spores survived, a survival rate of ∼7%. A representative set of spore colonies derived from 10 tetrads from this cross is shown in Figure 5. Among the five surviving sec14Δ cki1Δ hac1Δ spore colonies, four gave rise to small colonies, one of which is shown in Figure 4. One of the sec14Δ cki1Δ hac1Δ colonies failed to propagate after it was restreaked on YE PD medium. Out of the total of 67 tetrads, only one of the five surviving sec14Δ cki1Δ hac1Δ segregants exhibited apparently normal growth.

sec14 mutations confer tunicamycin sensitivity: Muta
tions in the UPR pathway are known to confer sensitivity to tm, which compromises the N-linked glycosylation process and leads to the accumulation of unfolded pro-
ch sch+ ch ch+ ch c c ch c ch c ch c c c c

Figure 5.—Genetic interaction between sec14Δ and hac1Δ mutations. Tetrad from the cross between the sec14Δ chi1Δ (HGY136) and chi1Δ hac1Δ (HGY006) strains were dissected and incubated for 4 days at 30°C and the genotypes of the individual spores were subsequently determined. A photograph of spore colonies derived from 10 representative tetrads is depicted. The genotypes corresponding to the individual spore columns are shown in the box below a photograph of the colonies, using the following abbreviations: c, chi1Δ; ch, chi1Δ hac1Δ; sc, sec14Δ chi1Δ; and sch, sec14Δ chi1Δ hac1Δ. The symbol * indicates a rare surviving sec14Δ chi1Δ hac1Δ spore from the cross. The genotypes of dead colonies (†) were deduced.

In the ER (Cox et al. 1993). The apparent synthetic lethality involving UPR and sec14 mutations led us to explore the tunicamycin sensitivity of strains carrying sec14Δ and sec14Δ mutations. Wild-type, hac1Δ, chi1Δ, and sec14Δ chi1Δ strains were tested at 30°C for growth on YEPD medium containing 1 mM tunicamycin (YEPD + tm). As expected, the wild-type strain grew normally on YEPD + tm, while the hac1Δ strain was tm sensitive (Figure 6).

No growth defect was observed in the chi1Δ strain grown on tm-containing medium (Figure 6). However, the sec14Δ chi1Δ strain exhibited a previously unreported sensitivity to tm (Figure 6), a phenotype that was further analyzed in the sec14Δ conditional mutant by examining growth on YEPD + tm medium at 25°C, 30°C, and 37°C (Figure 6). The sec14Δ strain exhibited a temperature-sensitive phenotype and failed to grow at 37°C, as previously reported (Novick et al. 1980; Bankaitis et al. 1989), and grew normally at 25°C and 30°C, regardless of the presence or absence of tunicamycin (Figure 6). The sec14Δ chi1Δ strain grew normally in the presence of tunicamycin at the sec14Δ permissive and semipermissive temperatures of 25°C and 30°C, but was very sensitive to tm at the sec14Δ restrictive temperature of 37°C (Figure 6), suggesting that inactivation of Sec14p leads to tunicamycin sensitivity.

sec14 chi1 strains exhibit elevated UPRE expression:
The unexpected finding of synthetic lethality between sec14 and UPR mutants and tunicamycin sensitivity in sec14Δ chi1Δ strains led us to examine UPR induction in sec14Δ chi1Δ strains. The fusion reporter construct UPRE-CYC-lacZ has been used previously to measure expression levels driven by the UPR responsive element, UPRE (Cox and Walter 1996). As expected, wild-type and chi1Δ strains exhibited low levels of UPRE induction when grown in YEPD medium (Figure 7). The UPR mutants, ief1Δ and hac1Δ, exhibited low levels of UPRE expression, similar to uninduced levels observed in the wild-type strain grown at 30°C, as described previously by Cox and Walter (1996). However, the sec14Δ chi1Δ strain, even at the sec14Δ semipermissive temperature of 30°C in YEPD medium, exhibited an induction of the UPRE reporter construct that is 3-fold higher than that of the wild-type or the chi1Δ strain grown under the same conditions (Figure 7). When the sec14Δ chi1Δ strain was grown at 37°C, the restrictive temperature for the sec14Δ allele, UPRE expression was ~6-fold higher than in the wild-type strain grown under the same conditions (Figure 7). In the sec14Δ chi1Δ strain, UPRE expression was 13-fold higher than in wild type at 30°C and 15-fold higher at 37°C (Figure 7). These results suggest that sec14 chi1Δ cells experience stress, which correlates with the in-
Figure 7.—The UPR is induced in sec14Δ kilΔ (JPV110) and sec14Δ kilΔ (SHY630) strains. A schematic diagram of pJC104 is shown above the graph. Plasmid pJC104 contains a fusion of four UPRE elements derived from KAR2, a minimal CYC1 promoter element, and the coding sequence for the E. coli β-galactosidase enzyme. Yeast cells of the indicated strains were transformed with the UPRE-CYC-lacZ reporter construct carried on pJC104, and fresh transformants were cultured for 7 hr at the indicated temperatures. β-Galactosidase activity was measured as described in materials and methods. The β-galactosidase activity unit was defined as OD₄₂₀/min/ml.

Increased sensitivity of sec14Δ kilΔ and sec14Δ kilΔ cells to tunicamycin and with the synthetic lethality of sec14 and UPR mutations.

**sec14Δ kilΔ, sec14Δ ire1, and sec14Δ hac1 cells exhibit abnormal vacuolar morphology:** Induction of UPRE in sec14Δ kilΔ and sec14Δ kilΔ cells suggested that they experienced abnormal stress, leading us to examine their subcellular morphology. Wild-type, ire1Δ, hac1Δ, sec14Δ, kilΔ, sec14Δ kilΔ, sec14Δ ire1Δ, and sec14Δ kilΔ hac1Δ cells were examined using electron microscopy. Wild-type and hac1Δ cells were found to have similar normal morphology at 25° and 37° (Figure 8, A–F) and ire1Δ cells were similar, except that the vacuole seemed to be slightly enlarged compared to the wild-type strain (Figure 8, E and F). At 25° and 37°, the kilΔ strain exhibited normal subcellular morphology similar to that observed in the wild-type strain (data not shown).

However, regardless of the presence of the kilΔ suppressor, severe defects in subcellular morphology (Figure 8, G, H, J, L, N, P, and R) appeared upon sec14Δ inactivation. Subcellular morphology of the sec14Δ, sec14Δ kilΔ, sec14Δ kilΔ ire1Δ, and sec14Δ kilΔ hac1Δ strains appeared to be normal when the cells were grown at the sec14Δ permissive temperature of 25° (Figure 8, I, M, O, and Q). However, defective subcellular morphology became evident in sec14Δ, sec14Δ kilΔ, sec14Δ kilΔ ire1Δ, and sec14Δ kilΔ hac1Δ strains within 1 hr following a shift to the sec14Δ restrictive temperature of 37°. An elevated population of large (250–500 nm) membrane-bound structures, possibly enlarged Golgi, was detected in the sec14Δ kilΔ cells within 2 hr following the temperature shift (Figure 8L). Similar defects were observed in all strains carrying sec14Δ mutations and were not correlated with ire1Δ, hac1Δ, or kilΔ mutations. Interestingly, vacuolar structure defects were prominent...
in the sec14Δ cki1Δ strain. The sec14Δ cki1Δ cells also exhibited severe fragmentation of the vacuole at 25°C (Figure 8G). However, the fragmented vacuolar morphology of the sec14Δ cki1Δ strain appeared different from the defect observed in the sec14Δ cki1Δ strain at 37°C. The vacuoles of the sec14Δ cki1Δ strain grown at 37°C looked invaginated (Figure 8, J–L). We have no independent evidence on what processes are occurring with the vacuoles of these cells.

**Activation of the UPR is not sufficient to fully derepress the INO1 gene in the presence of exogenous inositol:** Cox et al. (1997) suggested that activation of the UPR in inositol-deprived cells might be related to, or required for, the mechanism for induction of INO1 transcription in the absence of inositol. The fact that UPR expression is elevated in sec14Δ cki1Δ cells (Figure 7), which also express high levels of INO1 (Figure 4), is consistent with this hypothesis. However, the fact that INO1 is over-expressed in sec14Δ cki1Δ hac1Δ cells appears to negate this hypothesis. To further explore the relationship between UPR induction and INO1 expression, wild-type cells transformed with the UPRE-CYC-lacZ or the INO1-CYC-lacZ reporter gene were exposed to various types of stress, including heat, hypo-osmotic, hyperosmotic, ethanol, and oxidative stress. Cells were also shifted to I- containing 1 mM tunicamycin, conditions under which induction of UPRE-CYC-lacZ has previously been reported (Cox et al. 1997).

As expected, cells grown in I- medium exhibited very low levels of UPRE and INO1 expression (Figure 9). Consistent with the report by Cox et al. (1997), wild-type cells exhibited a 4.8-fold higher induction of UPRE when grown in I- medium than when grown in I+ medium. However, this effect was not observed until 3 hr follow-
a shift of wild-type cells from 25° to 37°, but this effect had almost disappeared by 3 hr (Figure 9). UPRE-CYC-lacZ was not significantly induced under hyper- (0.3 m NaCl) or hypo-osmotic (0.4 m sorbitol) stress or the oxidative stress imposed by the presence of H₂O₂ (0.4 mM; data not shown).

Despite the significant induction of UPRE under a number of the stress conditions described above, insignificant, or very minor, induction of the INO1 reporter gene was observed in I⁻/H11001 medium under all of these conditions, including the presence of 7.5% ethanol and 1 mM tunicamycin (Figure 9). Wild-type cells grown in I⁺/H11001 medium containing 1 mM tunicamycin exhibited minor induction of INO1 in comparison to the dramatic induction of UPRE. The level of INO1 induction in wild-type cells under these conditions is also insignificant compared to INO1 induction in cells shifted to I⁻ medium (Figure 9). These findings are consistent with the conclusion that the activation of the UPR is not sufficient to derepress INO1 transcription in the presence of inositol. Furthermore, activation of UPR does not appear to underlie the mechanism of INO1 activation during inositol deprivation, nor is an active UPR necessary for this regulation.

**DISCUSSION**

Transcription of INO1 and other UASᵣᵦᵣ-containing genes is regulated by the availability of exogenous inositol and is also affected by a signal generated from alteration of phospholipid metabolism (Patton-Vogt et al. 1997; Henry and Patton-Vogt 1998). In this report, we have examined the role of the UPR in transmitting the signal generated by altered phospholipid metabolism in the sec₁₄ cₖᵢ₁ genetic background, as well as the signal generated by the presence or absence of inositol. Cox et al. (1997) reported that a functional UPR is necessary for sustained expression of INO1 in the absence of inositol, but not for its initial derepression. Our examination of INO1 expression and PI synthesis in hac₁Δ and ir₁Δ cells shifted to I⁻ medium is consistent with this interpretation. However, the residual INO1 expression in hac₁Δ and ir₁Δ cells is still regulated in response to inositol, and we conclude, therefore, that a functional UPR is not necessary for the transmission of the signal controlling INO1 transcription in response to inositol.

Moreover, unlike the ir₁Δ and hac₁Δ single mutants, both sec₁₄₀ cₖᵢ₁ Δ ir₁Δ and sec₁₄₀ cₖᵢ₁ Δ hac₁Δ strains were able to grow without exogenous inositol at 25° and 30° (Figure 2A) and exhibited an Opi⁻ phenotype similar to sec₁₄₀ cₖᵢ₁ Δ strains at the semipermissive temperature of 30° (Figures 3 and 4). The expression of the INO1 reporter gene was elevated compared to wild type in sec₁₄₀ cₖᵢ₁ Δ hac₁Δ and sec₁₄₀ cₖᵢ₁ Δ ir₁Δ cells shifted to I⁻ medium at 30°, confirming that derepres-
sion and overexpression of the INO1 gene in the sec14Δ
ckiΔ genetic background does not require a functional
UPR (Figure 4). The Opi− phenotype and INO1 overex-
pression in the sec14Δ ckiΔ strain has been shown to be
due to elevated PC turnover caused by activation of
Pld1p (Patton-Vogt et al. 1997; Sreenivas et al. 1998).
Thus, the UPR pathway is not essential for INO1 tran-
scription in response to elevated turnover of PC in the
sec14Δ ckiΔ genetic background. Clearly, the signal gen-
erated by the altered phospholipid metabolism in the
sec14Δ ckiΔ genetic background is not transmitted via
the UPR pathway. Moreover, consistent with the results
obtained using the hacΔ single mutant, INO1 is not
expressed in the presence of inositol at 25° or 30° in
the sec14Δ ckiΔ hacΔ triple mutant, confirming that the
signal controlling INO1 expression in response to the
presence or absence of inositol does not require a func-
tional UPR (Figure 4).

Role of the UPR in INO1 expression: The analysis of
INO1 expression and regulation in hacΔ, ire1Δ, sec14Δ
ckiΔ ire1Δ, and sec14Δ ckiΔ hacΔ cells described above
indicates that a functional UPR pathway is not necessary
for INO1 expression or regulation. Despite previous re-
ports to the contrary (Cox et al. 1997; Hyde et al. 2002),
our results do not support the conclusion that UPRE
induction is sufficient to fully activate INO1 tran-
scription, at least when inositol is present in the medium.
In wild-type cells grown in I+ medium containing tunic-
camycin, we observed that UPRE was dramatically in-
duced, while INO1 expression was minimally affected
(Figure 9). Other conditions such as 7.5% ethanol treat-
ment that resulted in dramatic induction of transcrip-
tion of UPRE transcription had little or no effect on
INO1 expression when inositol was present (Figure 9).
Moreover, the INO1 reporter gene was induced earlier
and to a greater extent than the UPRE reporter gene
in wild-type cells shifted from I+ to I− medium, sug-
gesting that UPRE induction is not causally related to
INO1 activation under these conditions (Figure 9).

Nevertheless, it is clear that the level of INO1 expres-
sion is influenced by the HAC1 and IRE1 gene products.
The failure to sustain INO1 expression (Figure 4) and the
consequent effect that this has on phospholipid
metabolism (Table 2) explains the inositol auxotrophy
of hacΔ and ire1Δ strains. Yet, the mechanism by which
the HAC1 and IRE1 gene products influence INO1 ex-
pression remains elusive. Our results suggest that the
effect of the UPR on INO1 expression must be somewhat
indirect, since INO1 transcriptional derepression in I−
medium precedes UPRE induction in cells shifted to I−
medium and UPRE induction does not elicit high levels
of INO1 transcription in cells supplied with inositol (Fig-
ure 9). Mori et al. (2000) reported that Hac1p produced
from an unspliced form of HAC1mRNA is able to sup-
press inositol auxotrophy of hacΔ mutants. However,
they also demonstrated that Hac1p produced from the
spliced form of HAC1mRNA is a more efficient tran-
scription factor for UPRE than Hac1p produced from
the unspliced form of HAC1mRNA (Mori et al. 2000).
The results of Mori et al. (2000) suggest that once Hac1p
is produced, regardless of which form, it not only func-
tions as a transcription factor for the UPRE-containing
genes, but it also plays a separate, as yet undefined, role
in sustaining a high-enough level of INO1 expression
to alleviate the inositol auxotrophy of hacΔ cells. Thus,
it may be that it is the production of Hac1p, per se,
rather than the induction of the UPRE, that is critical
for sustained INO1 expression.

A functional UPR is required for the survival of sec14
ckiΔ strains: An unanticipated finding of this study was
the involvement of the UPR in survival of sec14Δ ckiΔ
strains following Sec14p inactivation. Both sec14Δ ckiΔ

![Figure 9](https://example.com/figure9.png)

**Figure 9.**—Comparison of induction kinetics of the INO1 and UPRE reporter genes in wild-type cells grown under various stress conditions. Wild-type strain SH629 was transformed with pJH359 (INO1-CYC-lacZ) or pJC104 (UPRE-CYC-
lacZ). Transformants were precultured in I+ medium at 30°. Wild-type transformants were then shifted to stress conditions including inositol de-
privation (I− medium at 30°), ER stress (I+ containing
1 mM tm at 30°), ethanol treatment (I+ containing 7.5%
ethanol at 30° in the presence of 2% glucose), and heat shock (I− at 37°), and
I− medium at 30° was used as a control. Samples
were taken at 1 and 3 hr following the shift and
analyzed for β-galactosidase activity as described
in MATERIALS AND METHODS. The β-galactosidase
activity unit was defined as OD420/min/ml.
and sec14Δ cki1Δ strains exhibited high levels of UPRE-CYcLacZ transcription relative to the wild-type or cki1Δ strain (Figure 7), indicating that the UPR is induced when Sec14p is inactivated. The tunicamycin sensitivity of the sec14Δ cki1Δ strain grown at 37°C (Figure 6) suggests that stress induced by treatment with tunicamycin and by inactivation of Sec14p may be additive, since the combination of the two is lethal.

Moreover, sec14Δ cki1Δ ire1Δ and sec14Δ cki1Δ hae1Δ cells do not grow at the sec14Δ restrictive temperature of 37°C (Figure 2, A and B), and the triple deletion mutants are not viable (Figure 5). Activation of the UPR pathway appears to be essential, therefore, in enabling the cell to deal with the stress that is correlated with the aberrant cellular morphology observed in sec14 cki1 strains (Figure 8). Among the UPR target genes in yeast identified by Travers et al. (2000) are genes involved in secretory function, including ER quality control and vesicle trafficking. Thus, the essential role of the UPR in survival of the sec14 cki1 strains may be to trigger upregulation of genes whose products are involved in vesicle trafficking or ER quality control under the stress condition produced when Sec14p is absent or inactivated.

The process of ER-associated protein degradation (ERAD) has been reported (McCracken and Brodsky 1996) to be regulated by the UPR under ER stress (Friedlander et al. 2000; Ng et al. 2000; Travers et al. 2000). Travers et al. (2000) suggested that the UPR induces transcription of genes involved in ERAD. Mutations in ERAD not only lead to activation of the UPR, but are also synthetically lethal in combination with mutations in the UPR (Friedlander et al. 2000; Ng et al. 2000; Travers et al. 2000). The negative genetic interaction of ERAD and UPR mutations resembles the synthetic lethality of UPR mutations and sec14Δ mutations that we observed in the cki1Δ background (Figures 2 and 5). Vashist et al. (2001) reported that ERAD substrates are either sorted in the ER for retention or transported to the Golgi via COP-II-coated vesicles and retrieved back to the ER via the retrograde transport system for proper degradation via ERAD. Vashist et al. (2001) further suggested that membrane transport between the ER and Golgi is required for ER quality control. The role of the UPR in upregulation of genes involved in ERAD and vesicle trafficking (Travers et al. 2000) and the synthetic lethality of ERAD and UPR mutations (Ng et al. 2000) suggest that the UPR plays an essential role in maintaining ER homeostasis under ER stress by enhancing both ERAD and membrane trafficking.

Consistent with the idea that membrane trafficking and the UPR pathways are functionally independent, a mammalian substrate of unconventional splicing by Ire1p, XBP-1 mRNA (Yoshida et al. 2001; Calfon et al. 2002), was recently discovered and its product has been postulated to play an important role in liver development as well as the secretion of immunoglobulins in B lymphocytes (Reimold et al. 2000, 2001).

Whatever the mechanism by which UPR and membrane trafficking pathways interact with each other, the results reported here indicate that the inactivation of Sec14p activates the UPR and that the UPR is essential to sec14Δ bypass suppression by cki1Δ.

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